RECOVERY OF STREPTOMYCIN FROM AQUEOUS SOLUTIONS USING FIXED BED ION EXCHANGE TECHNIQUE

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RAJENDRA MISRA

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CERTIFICATE

It is certified that this work has been carried out under my supervision and that this has not been submitted elsewhere for a degree.

9th March 1973

(Prof.) M. Gopala Rao, Ph.D. (Wash.)
Associate Professor of Chemical Engineering
Indian Institute of Technology
Kanpur-208016, India

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Author

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ABSTRACT

Preliminary investigations have been made to study the equilibria and kinetics of the sorption of streptomycin from aqueous solutions by weakly acidic cation exchange resins, KB-4P-2 (Russian) and IRC-50 (American). The capacities of the resins are given below:

Resin	Capacity in meq/gm dry resin in hydrogen form	
	Hydrogen-Sodium	Sodium-Streptomycin
KB-4P-2	12.9	2.445
IRC-50	10.9	2.400

As the capacities in streptomycin form are considerably lower than the capacity of the resins in sodium form; it can be concluded that all the exchange sites are not accessible for the streptomycin ions.

The equilibrium studies have shown that the exchange equilibria for the sorption of streptomycin by the sodium form of the resins is highly favourable.

The kinetic studies of streptomycin sorption have been made in the ranges of the following variables; particle size 0.0562 cm - 0.1289 cm; Velocity 19.3 cm/min-3.62 cm/min; bed heights 7 cm - 13 cm.

The following empirical equation has been proposed to correlate the breakthrough data between the range of variables studied; $I = -8.06118\ dp - 0.02637F + 0.49332h - 2.13961$.

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CHAPTER I

INTRODUCTION

Streptomycin ($C_{21}H_{39}N_7O_{12}$) was first produced by Schatz and Waksman (1) in 1944. It was found out to be a tri basic laevorotatory molecule. Streptomycin is unstable in the pH range 3-7. However its salts with inorganic acids are most stable in the pH range 4-5.8(2), with the exact value depending on temperature, and are extremely soluble in water and insoluble in organic solvents. More particularly streptomycin sulphate is most stable at a pH of 4.5 (3).

Streptomycin exhibits antibiotic activity against a variety of gram-negative bacteria including Typhosa, Haemophylus Influenzae etc. The most important and most valuable feature of streptomycin is its high activity against the Tubercle Bacillus. Although streptomycin also exhibits sufficient anti bacterial activity against some gram positive bacteria, it is usually less effective in this respect than the Penicillins.

Streptomycin is usually toxic to animal tissues. A major difficulty in the use of streptomycin is the readiness with which the organisms become resistant to it. A brand of streptomycin sulphate for veterinary use in treatment of calf scours, enteritis of swine, dog and quail has been formulated by the Merck Company.

The antibiotic is produced by certain strains of the earthmold organism "Streptomyces Grieseus" when grown under suitable conditions. A solution of the suspension of nutrients composed of Glucose, Peptone, Soyabean flour or meat extract, salt and water is sterilized by heating at 120°C and then inocculated by a selected species of the genus "streptomyces". The organism is allowed to grow for four to five days under controlled conditions. During this time, the medium attains the consistency of a thick soup while the antibiotic is produced. The culture fluid is filtered under controlled conditions. The antibiotic is isolated and recovered from the broth; high purity and good yield at the lowest possible cost are some of the considerations in the design of the separation unit.

During its early production history e.g.; Merck's $3.5 million plant producing 100,000 gms per month in 1946 (4), the antibiotic was recovered from the fermentation broth on special carbons from which it was eluted with copious quantities of acid and water.

The availability of synthetic porous carboxylic cation exchange resins in 1948 revolutionized the streptomycin industry. Streptomycin, a bivalent cation in alkaline media (5) is adsorbed from the clear neutral filtrate on a porous carboxylic cation exchanger in sodium form. Complexing agent such as Sodium Ethylene Di amine Tetro Acetic Acid or Sodium Tri Poly Phosphate is added to the broth to eliminate competition from inorganic cations such as calcium and Magnesium

for the exchange sites.

These resins adsorb large amounts of streptomycin quite selectively and the antibiotic is eluted quantitatively as a highly purified concentrate with a small amount of acid. The ash from the elute is removed by adsorption on a highly cross-linked sulphonic acid cation exchange resin in hydrogen form and anion exchange resin in hydroxyl form. Low porosity of such cation exchangers aids in sieving out larger streptomycin molecules selectively. The eluted solution is decolorised by an activated charcoal treatment or a treatment with resins like Wolfatit - EW (6). The decolorised solution then goes for spray drying at 130°C to produce a white powder of the streptomycin salt.

A total of 80,000 K.G. of streptomycin was produced in India in 1972, the value of which is Rs.12 crores. The retail market price (1972) of streptomycin in India was Rs.1.5 per gram. A list of manufacturers of streptomycin in India is given in the Appendix A-1.

1.1 Aim and Scope of the Present Work:

The present study was started with a view to obtain data for the design of fixed bed ion exchange systems for the recovery of streptomycin from aqueous fermentation broth. In this connexion it was decided to study the characteristics of streptomycin adsorption equilibria on KB-4 P-2, a carboxylic cation exchange resin. Equilibrium data was also obtained for Amberlyte IRC-50.

An attempt was also made to use Zeokarb-216, a phenolic resin produced in India by Ion Exchange (India) Ltd. Zeokarb-216 is stable upto a pH of 9 and as the carboxyl groups dissociate only above pH-7, the exchange takes place at a pH greater than 7. The operating range for this resin in this case is therefore between the pH of 7 and 9. It was found difficult to mantain the pH of the solution between 7 and 9, and hence the idea of exploiting Zeokarb-216 for the recovery of streptomycin was abandoned. Zeokarb-226, which is an equivalent of IRC.50, could also be studied, but since the production of Zeokarb-226 by Ion Exchange (India) Ltd. was stopped in 1971, the design data for Zeokarb-226 system could not be obtained.

Since the resin is in sodium form before its loading with streptomycin, ion exchange equilibria between sodium and streptomycin ions will be necessary at a streptomycin concentration of about 4,000 U/ml in solution which is about the same as the concentration of streptomycin in the filtered broth.

The two resins used for equilibrium study are (i) KB-4 P -2 which is of Russian origin, and (ii) IRC-50 which is produced by M/S Rohm and Haas Co. Philadelphia, Pa. U.S.A.

One of the aims of the present study was to determine the capacities of the two resins for streptomycin loading in a fixed bed. Another objective was to obtain the break through curve for the sorption of streptomycin ions on KB-4 P -2 resin beds of two different particle size ranges. It

is necessary to obtain the rate data at a streptomycin sulphate concentration of about 4000 U/ml.

The scope of the work is in that the data obtained in the present study could be utilized for the design of an ion exchange column for streptomycin loading from aqueous solutions. The data could also be used for comparing the performance of any other new resins before their commercial exploitation.

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CHAPTER II

LITERATURE REVIEW

Most of the work done on the recovery of streptomycin from aqueous solutions appears in literature in the form of patents. There are some references in published literature also. In view of this, the present chapter is organized in the following manner: A summary of patent literature is presented first. Published literature is described in the second section. Literature on the chemical analysis of streptomycin is given in the last section.

2.1 Patent Literature:

The first process utilizing carboxylated cation exchange resins for the separation of streptomycin from clarified fermentation broth was patented by Taylor(7). Howe and Putter (8) compared various adsorbants like silicate exchange resin, sulphonated coal resin, phenol formaldehyde resin, Fuller's earth etc in respect of their capacity for loading and efficiency of recovery of streptomycin with that of the copolymers of Methacrylic acid cross-linked with five per cent and ten per cent DiVinyl Benzene. They described the process also giving full details. Resin copolymerised with five per cent Di Vinyl Benzene was shown to have the highest capacity 600-1500mg/g adsorbant. A modified version of their process appeared in another patent (9).

Peck (10) developed a chromatographic method for the purification of streptomycin. Pfizer Chas and Co. Ltd. (11) patented in 1968 a detailed process for the purification and concentration of streptomycin using Amberlyte IRC-50 and Dowex 50W X 16 resins. The final eluate contained one per cent ash and fifty ppm Calcium which represents a high purity for parenteral pharmaceutical use.

Katarzyna (12) described a non-ion-exchange process. In this process streptomycin was purified by heating under normal or reduced pressure and by adsorption of the decomposition products of the contaminants, on active carbon. Nager (13) used IRC-50 to load streptomycin from fermentation broth. The process was used to purify streptomycin in a single one bed ion exchange column and avoided the losses of repeated transfers. He later modified his process in 1970 (14).

Nitealia (15) treated the concentrated solution with 0.5 to 3% sodium bi sulphite to limit the activity of undesired micro-organisms in streptomycin culture liquid after fermentation, filtering and adding to a carboxy resin. Braune and Werner (16) prepared streptomycin sulphate of 99-100% purity from the methanolic double salt, streptomycin chloride-calcium chloride, by fractional precipitation with Di Ethyl Ammonium Sulphate.

Erik (17) adsorbed streptomycin on IRC-50 from unfiltered fermentation broth by a continuous upward flow, while the

column kept oscillating horizontally around its axis. In this process several columns may be mounted around a common axis to render the process continuous, running on a merry go round basis. Yudin (18) precipitated streptomycin sulphate from Butyl alcohol to obtain a free flowing salt preparation.

Shiro (19) described a method for the removal of insoluble substances from fermented streptomycin solution by the adjustment of pH and temperature during filtration. Takagi (20) patented a method for the decolorisation of streptomycin solution.

Kubota (21) prepared a phenol-formaldehyde resin having carboxylic groups and used it for refining of streptomycin. The resin had a liberation efficiency of 94.8%.

2.2 Published Literature:

Strong (22) described a Chromatographic method for the purification of streptomycin over Alumina column. Cartland(23) reported that streptomycin was precipitated fractionally with acetone from a methanolic concentrate at pH 2-8. He also gave a method to precipitate a streptomycin-calcium chloride double salt from methanolic solution of streptomycin chloride.

In the reports of Science Research Institute (Japan)(24) a new Phenol-Formaldehyde resin was described which was shown to have a high efficiency for adsorption and desorption of streptomycin from aqueous solutions. Fujita (25) studied

the effect of streptomycin concentration on Phenoxy acetic acid type cation exchangers.

Boiko (26) determined a process for the separation of the antibiotic streptomycin from culture media without the removal of mycelium. He also determined the optimum flow rate of the solution through the described column as 80-100 ml/gm resin. hr. Calcium ions in the culture broth were precipitated by the addition of 8-10% sodium oxalate.

Savitskaya and Bruns (27) investigated the adsorption from methanolic streptomycin chloride by weakly cross-linked carboxylic cation exchangers in the Lithium and Hydrogen form. In Methanol-Water solutions the adsorption attained a pseudo-equilibrium in contrast to a true equilibrium obtained in aqueous solutions. Desorption was achieved with Methanol-Hydrochloric acid solution.

Savitskaya (28) concluded that the formation of equilibrium state during adsorption of large organic cations of streptomycin, on carboxy cation exchangers KB-4 and KB-2, was mainly influenced by the number of cross-links in the resin which decreased with swelling. Equilibrium in the strongly swollen resin was established more slowly in the hydrogen form. In the medium swollen resins, the equilibrium was not established and the addition of sodium ions increased the sorption of organic cations. The velocity of sorption of organic cations was slower than that of sodium ions and it increased with the swelling of the resin.

Belaya (29) determined the activation energies and diffusion coefficients for streptomycin adsorption by various carboxy cation exchangers in the sodium form. Dependence of these characteristics on temperature and cross-linkage was also determined.

A good account of streptomycin production and processing is given in Kirk Othmer Encyclopedia of Chemical Technology(30). Ermakov (31) designed an apparatus for adsorption-desorption of streptomycin which is an ion exchange filter of variable cross-section with drainage facilities. This equipment increased the supply of the solution on to the filter 2-3 fold, increased the yield upto 86% and decreased the work cycle time.

Bogatskii (32) suggested the use of sodium tri poly phosphate for the elimination of Magnisium ions, streptidine and streptobiosamine admixtures adsorbed together with streptomycin on the cation exchange resins KB-4 and KB-4 P-2.

Bruns (33) used the co-polymers of Di Vinyl Benzene with Methacrylic acid, Acrylic acid, Itaconic acid, and styrene vinyl Benzoic acid for adsorption of streptomycin. He stated that the trapping of large organic ions by the resins was not an equilibrium process, since by changing the conditions of desorption, a marked increase of the ion exchange may be achieved. The completeness of desorption depended on the ratio of streptomycin content to the sodium content of the resin.

Stremovskii (34) from his experimental investigation in Vses Inst. Anti., Moscow, established that the process of ion exchange sorption of streptomycin was limited by inner diffusion in the granules of Ionite. Belaya (35) determined the diffusion coefficients and activation energies of streptomycin sorption by the carboxylic KB-4 and KMDM-6 resins at various temperatures. The effect of cross-linkage on these characteristics was also studied.

Kotula (36) found that the ion exchange resins Wolfatit E, Wolfatit EW and Asmit-259 were most effective to adsorb colored contaminants from the streptomycin solution. He also determined the optimum conditions of flow rate and the diameter of resin grains.

2.3 Analysis of Streptomycin:

Activity of streptomycin is expressed in terms of units. According to Indian standards, pure streptomycin sulphate powder has a potency of 800 U/mg.

For the identification of streptomycin, two methods are given in British Pharmacepoeia. In the first method streptidine sulphate (mp 283°C) is separated from a methanolic sulfuric acid solution of streptomycin, which is recrystallised from its solution in Tri Nitro Phenol. In the other method streptomycin in an alkaline medium is hydrolysed and the product gives a violet color with acidic

Ferric Chloride solution. Another method (37) uses substituted Benzene Sulfonic acids to produce salts of streptomycin having characteristic melting points.

Quantitative Analysis:

The possibility of quantitative chemical analysis of streptomycin was first suggested by Spielman (38) and later corroborated by Solomon (39). Spielman indicated that an ultraviolet absorption at 274 m. micron of the Maltol produced by alkaline hydralysis of streptomycin was proportional to the activity of streptomycin in the preparations. John Seudi et al (40) tried to use modified Elgon-Morgan test of Gluocosamines for streptomycin. Their assay gave 400% of the biological potency of the sample at low concentrations.

Titus and Fried (41) used the Maltol test for the assay of streptomycin. Federal register (42) gave tests and methods for the assay of streptomycin sulphate. Blauchard and Buhle(43) exploited the interaction of the carbonyl group of streptomycin with a colored semi-carbazide and colorimetrically determined (at 580 m.μ) the streptomycin derivative thus formed.

Roux (44) got a red color by reacting alkaline sodium Nitro Prusside with streptomycin. The color turned yellow on heating and green upon the addition of excess acetic acid. This color could be used for the estimation of streptomycin. Buck and Mader (45) measured the absorbance of the red color

developed by streptomycin with 1-Naphthol and NaO Br at 520 m. micron. In this method guanidine group containing impurities interfere with the analysis.

Eisen and Bricker (46) distilled the Maltol produced by alkaline hydrolysis of streptomycin. The colours developed by Maltol with Ferric ions and Phenol reagent of Folin and Ciocaltoau were measured at 550 m. micron, and 775 m. micron respectively. Amperometric micro-titration using polarograph by Conn and Norman (47) used the dye formed by coupling of diazotized Para rosa aniline with 1-naphthol -4-sulphonic acid which precipitated streptomycin quantitatively. Davies et al (48) utilized the formation of characteristic zones in reeded agar column. Colorimetric measurements were made of the Formaldehyde liberated upon oxidation with per iodate.

Delaby and Stephan (49) used Nessler's reagent in Iodimetric titration of streptomycin. Kaern (50) reviewed the various physico-chemical methods for the determination of streptomycin in solutions.

Valedinskaya (51) compared the orange colors developed by adding alkaline sodium Nitroprusside to streptomycin solution for the estimation of streptomycin. Monastero (52) based his method of analysis on the measurement at 490 m. micron of the orange red color resulting from the addition of sodium nitroprusside and potassium Ferricyanide to a dilute aqueous solution of streptomycin sulphate. The

method is reported to be simple and reproducible. Savitskaya and Kartseya (53) reacted 2,4 dinitro phenyl hydrazine with streptomycin to produce a yellow hydrazone whose optical density at 4300 Å was a function of its concentration. Sugars and Amino sugars did not interfere in this analysis. Szaffir and Bunnet (54) adapted Voges Prauskauer reaction for a rapid and reproducible colorimetric assay of streptomycin. Activities as low as 25 U/ml could be determined by this method.

Fujiwara (3) used maltol method for colorimetric estimation of streptomycin. Barilari (55) gave a new method which was more cumbersome than the maltol method.

Maltol method was used in the present study for the assay of streptomycin. Transmittance measurements, of the violet color developed with Ferric ions, were made at 520 m. micron because at this wavelength of monochromatic light, the violet color was found to have maximum absorbance (Fig.1)

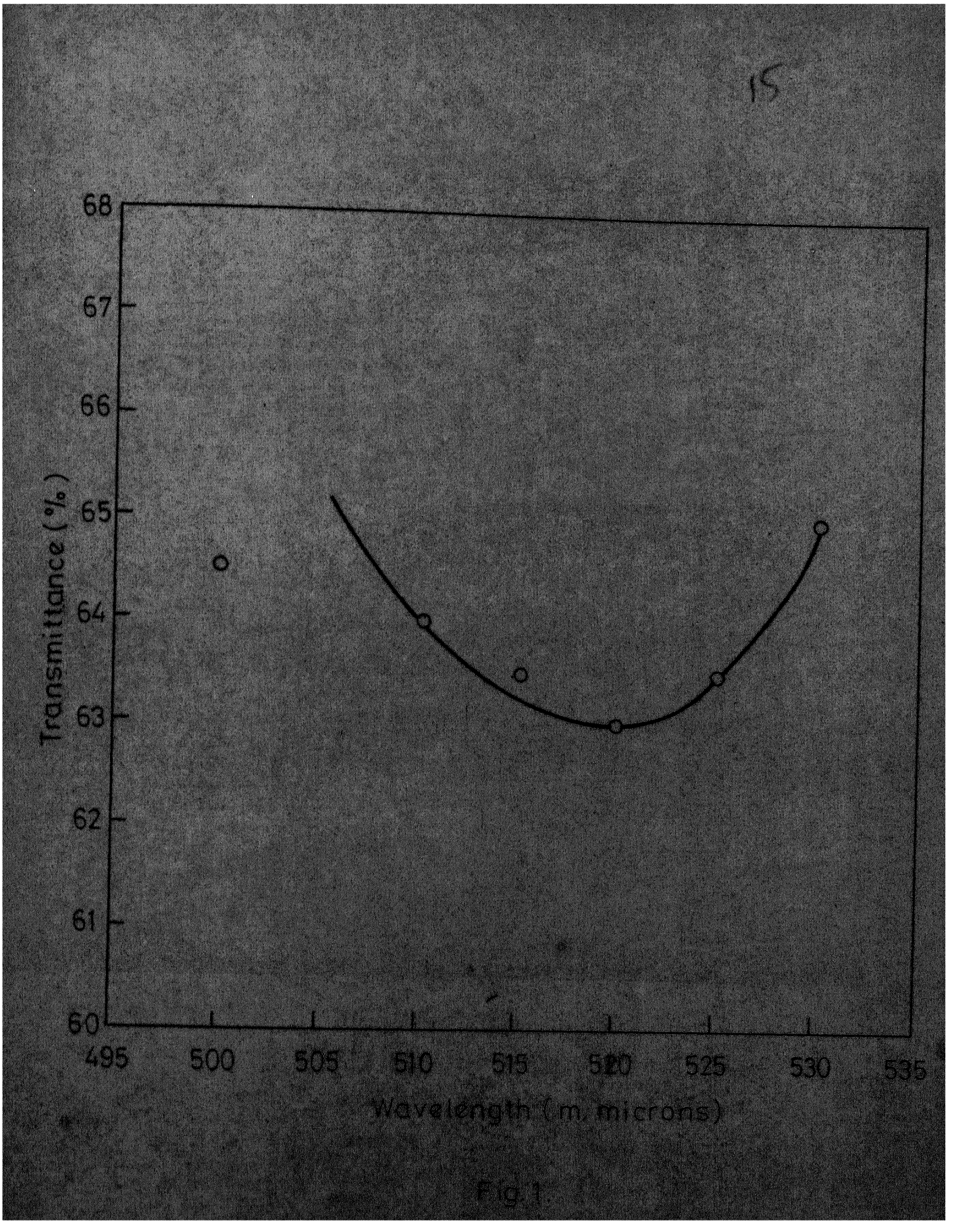

68
67
66
65
64
63
62
61
60
Transmittance (%)
495
500
505
510
515
520
525
530
535
Wavelength (m. microns)

Fig. 1

CHAPTER III

EXPERIMENTAL

Out of various methods available for quantitative analysis of streptomycin, the Maltol method was chosen for its ease of operation and accuracy of results. The method is described in detail later on in the chapter.

For the study of breakthrough phenomena in the kinetic studies, the deep-bed technique was employed in preference to the shallow bed method. In the shallow bed method, analysis of the effluent will be a major problem. Furthermore, for the measurements of effluent concentration very fast analytical method is required which is not available for streptomycin. Batch method was not used as it does not give any useful information regarding the effect of flow rate on the rate of transfer.

3.1 Materials and Equipment:

A corning glass graduated column made from 100 ml burette having a sintered glass disc fitted at the bottom was used for the studies. An inverted U tube was attached at the bottom to control the level of the solution above the top of the bed. To allow for back-wash, an outlet was provided in the upper portion of the column which could be sealed with a rubber stopper when needed. A constant flow

rate through the bed was maintained by using a pressure regulated aspirator bottle for holding the solutions as shown in Figure 2.

Fischer Titrimeter was used for the pH-metric titrations, and spectronic - 20 of Bausch and Lomb. for transmittance measurements. A Mettler balance for weight measurements, temperature controlled vacuum furnace for drying the resin and Taylor's screen for classifying resin particle sizes are some of the other equipments used in the study.

All chemicals used were of Analar grade. Streptomycin sulfate powder (activity 695 U/mg) was obtained from Indian Drugs and Pharmaceuticals Limited, Rishikesh.

Some of the properties of the ion exchangers used in the study are given below:

Origin	Name	Capacity	Na^{+} -- Streptomycin^{+++}	Particle Size	%	x linkage
USSR	KB-4P-2	12.9 meq/gm	2.445 meq/g (hydrogen form)	-36 + 80	90	2.5%
USA	IRC-50	10.9 meq/gm	2.40 meq/g (hydrogen form)	-30 + 80	90	4-5%

Both of the resins studied are weakly acidic cation exchangers.

3.2 Streptomycin Analysis:

Streptomycin is hydrolysed in an alkaline medium to produce Maltol which gives a violet color with Ferric ions. Logarithm of the transmittance of this color is directly proportional to the concentration of streptomycin if the color

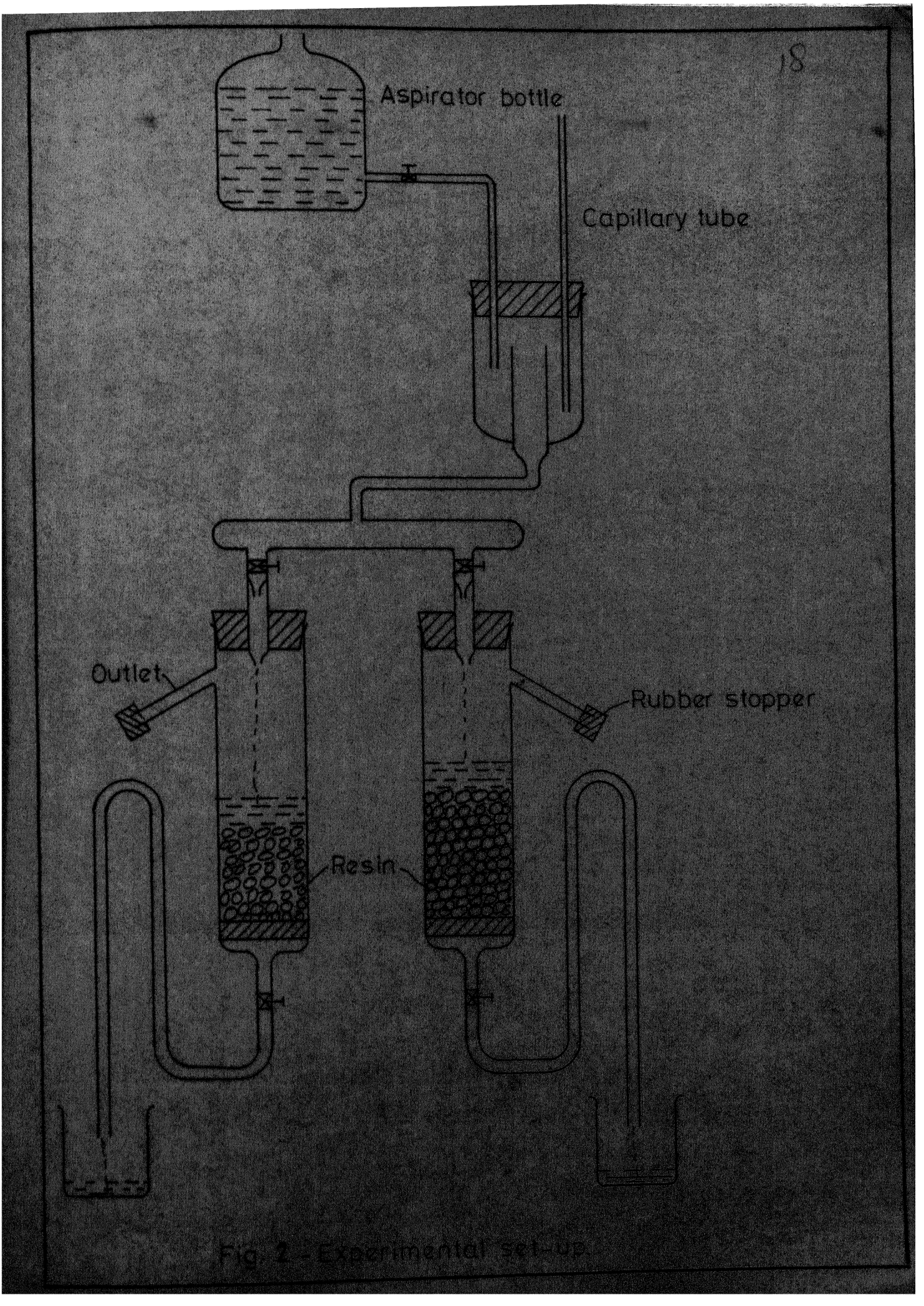


Fig. 2 - Experimental set-up.

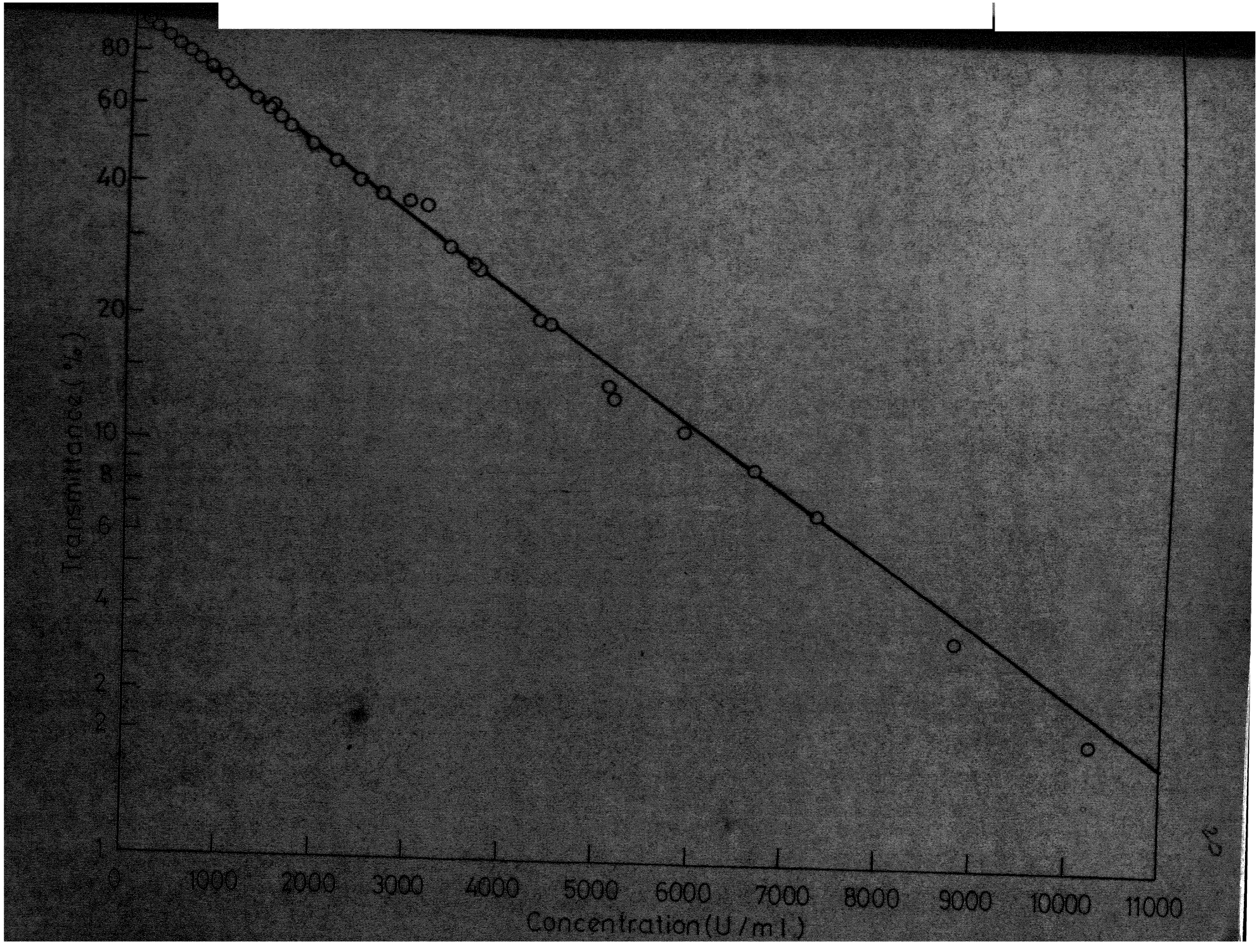
is produced under identical conditions of pH of the sample, amount of alkali, time, temperature of hydrolysis and the amount of Ferric ions used for developing the color.

A Russian standard-sample of streptomycin having an activity of 740 U/mg was used for determining the calibration curve. A standard solution of 1198.1 U/ml was prepared using the standard streptomycin sulfate powder. The stock solution was diluted to different concentrations with distilled water. The pH of the samples was found out to be around 5.3± .1.

One m.l. of the standard solution was taken in a test tube with the help of a pipette and madeup to seven m.l. with distilled water. 1.5 m.l of 0.5N sodium hydroxide from the stock solution was then added and the tube was heated in a water bath for five minutes after which the tube was cooled rapidly in ice cold water to quench the reaction. Two m.l. of one per cent Ferric ammonium sulfate solution, prepared in 0.5N sulfuric acid solution, was added into the tube. A violet color developed whose transmittance was measured with spectronic-20 at 520 m. micron. Such transmittance was measured for solutions of various concentrations and calibration curve (Fig. 3.) plotted

3.3 Conditioning and Regeneration of the Resins:

About 20 gms of resin (supplied in hydrogen form) was transferred to a glass column fitted with a sintered disc at the bottom and an outlet at the top. The resin was back washed


80
60
40
20
10
8
6
4
2
2
1
Transmittance (%)
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
Concentration (U/ml)

with water flowing at such a rate that fines were elutriated out. The bed was then washed with distilled water and a five per cent sulphuric acid solution was then passed through the bed at a slow flow rate till the Phenolphthalein end point of the effluent and influent against standard 0.1N sodium hydroxide solution was same. The resin was again washed with distilled water till the effluent was neutral to methyl orange.

A four per cent solution of sodium hydroxide was then passed through the column at a slow flow rate till exhaustion of the bed as indicated by the same alkali concentration of influent and effluent measured by titration against 0.1N oxalic acid. The bed was then washed with deionized water. The resin could be converted to hydrogen form with five per cent sulphuric acid solution and to sodium form with four per cent sodium hydroxide solution.

3.4 Determination of Solids Content in the Resin;

After conditioning to whichever form it was desired, the resin was transferred to a Buckner funnel where excess moisture was removed by drawing air through the funnel covered on top with filter paper using a Cenco hyvac pump. The adhering water was then soaked in filter papers. The free flowing swollen resin was then transferred to three petri-dishes, whose weights, when empty, were known, and weighed. The dishes were then kept in the temperature controlled vacuum oven and heated at 110°C and at a vacuum of 21 inches

mercury for drying the resin. The drying was continued till the resin attained a constant weight. A dessiccator containing calcium chloride was used to cool the hot dishes before weighing. Percent solids was then determined as given below. (Also in appendix: A.6.1)

$$\text{Percent solids} = \frac{\text{Weight of oven dried resin}}{\text{Weight of resin before drying}} \times 100$$

3.5 Capacity Determination:

A known amount of the hydrogen form of the swollen resin was taken in the column. Solids content of the resin from the same swollen lot was determined. A small amount of five per cent sulphuric acid was then passed through the column and then the bed was washed with deionized water till the effluent was neutral to Methyl orange.

1N sodium hydroxide solution was then passed through the column at a slow rate and 1 m l samples of effluent were collected at various through put volumes.till sodium hydroxide concentration of effluent and influent were same as determined by titration against standard oxalic acid solution. measured.
The total volumes of solution passed and collected were also/
The bed was then washed methyl orange
/with distilled water tilleffluent was neutral to/ and whole of the effluent made up to a certain volume with distilled water. A sample of the effluent was analysed for its alkali content. From the difference between the total alkali passed and that collected as effluent, the capacity was determined

as shown in sample calculation A.6.2.1 in the appendix.

A check was made by eluting the total sodium ions with five percent sulfuric acid and analysing for acid content of the influent and effluent with standardized sodium hydroxide solution using Phenolphthalein end point. Both the values agreed well.

For sodium $^{+}$ .... streptomycin $^{+++}$ exchange capacity, a known amount of the sodium form of the resin was taken in the column at a wash effluent pH of approximately 10. Streptomycin sulphate solution of pH slightly greater than 7 and concentration approximately 4000 U/ml was passed through the column at a slow flow rate till complete exhaustion of the bed. Samples at various through put volumes were also analysed for streptomycin concentration. Bed was then washed with deionised water.

Resin capacity for streptomycin loading was calculated from the difference between total streptomycin passed through the column and total streptomycin collected in the effluent.

Streptomycin was then eluted with five percent sulphuric acid solution passing through the column at a very slow rate (1-2 ml./min).Total streptomycin eluted was determined by chemical analysis. pH of the elute samples was increased to 5.3 by adding sodium hydroxide solution using Fischer titrimeter before going for alkaline hydrolysis of streptomycin.Streptomycin was analysed as mentioned earlier.

Capacity of the resin for streptomycin was obtained as below (Also in sample calculation A.6.2.2 in the appendix)

$$\text{Capacity } Q = \frac{\text{Volume of elute at pH5.3} \times \text{Concentration of elute in U/ml}}{\text{Solids content} \times \text{Weight of swollen resin}}$$

$$\frac{* \ 1000}{800{,}000 \ * \ 728.7} \qquad \frac{\text{milli equivalents}}{\text{gm dry resin in sodium form}}$$

Where each gram of pure streptomycin sulphate contains 800,000 units and 728.7 is the equivalent weight of streptomycin sulphate. Temperature was between 32°C and 36°C.

3.6 Equilibrium Procedure:

The equilibria of exchange between sodium and streptomycin ions was studied using columnar technique. A total anionic concentration of $\frac{N}{45}$ in equilibrating solutions was used for all the measurements. $\frac{N}{90}$ stock solutions of streptomycin sulphate and sodium chloride were prepared. Five equilibrating solutions with following ionic compositions were used.

Sl.No.	Streptomycin	Sodium
1	25	75
2	33.3	66.6
3	50	50
4	66.6	33.3
5	75	25

A known amount of sodium form of the resin placed in the column was equilibrated with the solution of known ionic composition by passing the solution through the bed at a slow flow rate (2-3 ml/min). The run was stopped when the streptomycin concentration in the effluent became equal to that in the influent. Then the bed was washed with deionised water and adsorbed streptomycin ions were eluted with five per cent sulfuric acid solution. Total quantity of streptomycin ions thus eluted was determined analytically. From the composition of equilibrating solutions, capacity of the resin and the corresponding value of streptomycin adsorbed, the values of C/C_0 and q/Q were calculated and plotted on an ordinary scale as shown in Fig. 4. Sample calculation (A.6.3) is given in appendix.

3.7 Procedure for Kinetic Study:

Same procedure as in the capacity determination was employed except that a constant flow rate of streptomycin sulphate solution of known concentration was maintained throughout over a bed of a known amount of resin of a given particle size in the sodium form. pH of the loading solution was brought to slightly greater than 7 by addition of a few drops of 0.1N sodium hydroxide solution.

Streptomycin concentration in the effluent was determined at various time intervals after the start of the run.

Two ranges of particle diameters namely average 0.0562 cm and 0.1289 cm in sodium form, five different flow rates for each particle size and for two different bed heights of each particle size were used to get breakthrough data on KB-4P-2 in sodium form.

For IRC-50 in sodium form breakthrough data, for only one bed height for a wide range of particle sizes at a single flow rate, was determined.

The room temperature was between 30 and 34°C for the kinetic studies.

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of the resins in fixed beds were measured. The value for the Russian resin in sodium form was 0.7 gm/ml whereas the value for the American resin was 0.82 gm/ml.

4.2 Capacity Measurements:

The results of capacity measurements are given in Appendix A.6-2. The capacity of the Russian resin in sodium form is 12.9 meq/gm. whereas the capacity of the American resin is 10.9 meq/gm. Again the capacity of Russian resin in streptomycin form is 1.8475 meq/gm. dry resin sodium form. It can be seen from the measurements that Russian resin has higher capacity. However, both the resins have only a fraction of their exchange capacity available for the adsorption of streptomycin. This could be due to all the active sites not being accessible for the large streptomycin cation. Perhaps a more porous resin with good physical properties could be developed for the streptomycin process. However as the cross linkage goes on decreasing, the strength of the resin particles also goes down.

4.3 Equilibrium Studies:

The results of the equilibrium studies are presented in Tables 3 and 4 and in figure 4. A pH of 8 was maintained in the solution phase. Equilibria of streptomycin - sodium exchange was measured. Both the resins have high selectivity for the streptomycin ion. The equilibrium isotherm is very nearly the irreversible type for KB-4P-2 and quite favourable for IRC-50. This result can again be expected as the streptomycin

ion is a bivalent cation.

4.4 Kinetic Studies: The kinetic studies have been made only with KB-4P-2 . Two different bed heights namely 7 and 12-13 cm., two average particle diameters of the resin viz., 0.1289 cm. and 0.0562 cm. and five flow rates were employed. The data are presented in Tables 6 to 10 and are shown in figures 5-9 The elution curve is as expected an S shaped curve. The breakthrough point in all the runs did not occur early and the performance of the ion exchanger was satisfactory. Data for the IRC-50 resin is presented in Appendix A-7, Figure 13.

Several correlations were tried to predict the break-through curve as a function of the variables investigated; if fluid film is controlling the mass transfer, and the equilibrium is irreversible type the following equation should correlate the data:

$$x = e^{u} \qquad (1)\ (56)$$

where

$$u = N_{fF}(z-1)-1 \qquad (2)$$

N_{fF} = Number of transfer units based on fluid phase

and

z = Through put parameter

However, if the particle phase diffusion is controlling the mass transfer, the following equations are available for the break-through curve in a favourable equilibrium case:

1. An exact solution for the irreversible constant pattern breakthrough is obtained from the results of Wicke (57) as

based on the rate relation

$$D_P \left(\frac{\partial^2 y_r}{\partial r^2} + \frac{2}{r} \frac{\partial y_r}{\partial r} \right) = \frac{\partial y_r}{\partial T} \qquad (3)$$

for perfectly spherical particles. Here D_P is the particle phase diffusivity, y_r is the dimensionless solid phase concentration at any internal radius r and T is the reaction time.

$$x = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} e^{-u} \qquad (4)$$

with $u = n^2 (\psi N_{PF}(z-1) + 0.64)$ (5)

N_{PF} = Number of transfer units based on the particle phase

and $\psi = \frac{\pi^2}{15}$

2. Glueckauf and Coates (58,59) have provided the solution of the Linear Driving Force Approximation, which, for the irreversible case is

$$x = 1 - e^{-u} \qquad (6)$$

where $u = N_{PF} (z-1) + 1$ (7)

3. The Vermeulen Quadratic Driving Force Approximation (60) has been integrated in the completely irreversible case to give

$$x = (1 - e^{-u})^{0.5} \qquad (8)$$

where $u = \psi N_{PF} (z-1) + 0.61$ (9)

Integral solutions implicit in x are available for partially irreversible cases also.

Data in the present study could not be correlated at all by equations for the fluid film control. However, the initial portion of the break-through curve and the break-through point itself could be predicted by the equation of the break-through curve for the fluid film control. When the equations for the particle phase diffusion control were employed, a constant resin phase diffusivity could not be obtained. However the latter portion of the breakthrough curve could be correlated by the linear driving force approximation of Glueckauf and Coates mentioned earlier.

Based upon the above equations 6 and 7 a graph was plotted between ln(1-x) and z, as shown in Figures 11 and 12. The slope of the straight lines, N_{PF} can be correlated as a function of flow rate.

The intercept is correlated by the equation given below:

$$I = -8.06118\ dp + (-0.02637)F + 0.49332h - 2.13961$$

The constants in the above equation were obtained using a Least squares regression program on the computer IBM 7044. The error between the value of intercept calculated from Figures 11 and 12 and that calculated using the proposed model are shown in the Table 12. It is evident that the model fits the data reasonably well.

The empirical equation proposed in the present study could be employed for the design of the ion exchange column

for the recovery of streptomycin .

As shown in the Appendix A-7 the regeneration of streptomycin form of the resin and the recovery of streptomycin from the resin phase, were not satisfactory(79-83%), further studies are required for finding the optimum conditions of regeneration.

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TABLE 1

No.	Wave length (m. microns)	Transmittance (%)
1	500	64.5
2	510	64.0
3	515	63.5
4	520	63.0
5	525	63.5
6	530	65.0

TABLE 2

CALIBRATION DATA

No.	Concentration (U/ml)	Transmittance (%)	No.	Concentration (U/ml)	Transmittance (%)
1.	119.8	96	16.	3354.7	29
2.	239.6	91	17.	3594.3	26.5
3.	479.2	83	18.	4313.2	20
4.	599.0	80	19.	5032.0	14
5.	718.8	76	20.	1462.2	60
6.	958.5	70	21.	2193.4	45
7.	1198.1	65	22.	2924.5	37
8.	1317.9	62	23.	3655.6	26
9.	1557.5	56	24.	4386.7	19.5
10.	1677.3	54	25.	5117.8	13
11.	1916.9	49	26.	5848.9	11
12.	2156.6	45	27.	6580.0	9
13.	2396.2	41	28.	7311.2	7
14.	2650.8	38	29.	8773.4	3.5
15.	3115.6	36	30.	10235.6	2

TABLE 3

EQUILIBRIUM DATA

Resin: KB-4P-2 Capacity: Q = 2.446 meq/gm dry resin in Hydrogen form

No.	M equivalents of streptomycin in solution phase per gram solvent	Equivalent fraction of streptomycin in solution phase $x = c/C_o$	M equivalents of streptomycin in resin phase per gram resin q	Equivalent fraction of streptomycin in resin phase per gram $y = q/Q$
1.	0.00748	0.3900	2.4268	0.9927
2.	0.01158	0.8800	2.4330	0.9952
3.	0.00366	0.3340	2.4226	0.9910
4.	0.00748	0.2150	2.3774	0.9725
5.	0.00158	0.9860	2.4392	0.9977
6.	0.00748	0.0320	1.5094	0.6170

TABLE 4

Resin: IRC -50 Capacity; Q = 2.4008 meq/gm dry resin in Hydrogen form

1.	0.00748	0.3900	2.0678	0.8588
2.	0.01158	0.8800	2.1108	0.8733
3.	0.00366	0.3340	2.0584	0.8544
4.	0.00748	0.2150	1.5232	0.6300
5.	0.00158	0.9860	2.2644	0.9400

TABLE 5

KINETIC DATA HYDROGEN-SODIUM EXCHANGE

Dry KB-4P-2 (Hydrogen Form) in the column = 5.5809 gms
Flow Rate = 5 ml/min

No.	Throughput Volume (ml)	Volume of oxalic acid used against 5 ml effluent(C)	$x = C/C_o$
1.	57	1.7 ml.	0.34
2.	75	4.4 ml.	0.88
3.	100	4.9 ml.	0.98
4.	105	5.0 ml.	1.00

Dry IRC-50 (Hydrogen form) in the column = 4.64 gms.
Flow Rate = 5 ml/min.

1.	30	0.25 ml.	0.005
2.	50	2.4 ml.	0.48
3.	80	4.4 ml.	0.88
4.	100	4.9 ml.	0.98
5.	130	5.0 ml.	1.00

TABLE 6

KINETIC DATA USING KB-4P-2
(Sodium-Streptomycin Exchange)

Run 1 ; Temperature = 34°C; Influent Concentration C_o = 3300 U/ml

Particle Diameter=0.1289 cm Bed Height = 7 cm. Flow Rate = 20 ml/min.					Particle Diameter = 0,0562 cm. Bed Height = 7 cm. Flow Rate = 20 ml/min.			
No.	Time (min.)	$x = C/C_o$	1-x	z	Time (min.)	$x = C/C_o$	1-x	z
1.	10	0.47	0.53	0.252	10	0,166	0.834	0.253
2.	30	0.85	0.15	0.755	30	0,742	0,258	0.758
3.	40	0.886	0.114	1.006	40	0.803	0.197	1.011
4.	50	0.932	0.068	1.258	50	0,894	0.106	1.264
5.	56	0.977	0.023	1.408	60	0.932	0,068	1,516
6.	60	1.000	0.000	1.509	70	1.000	0.000	1.769

TABLE 7

Run 2 ; Temperature = 34°C; Influent Concentration C_o = 3560 U/ml

Particle Diameter=0.1289 cm. Bed Height = 7 cm. Flow Rate = 32 ml/min.					Particle Diameter =0,0562 cm. Bed Height = 7 cm. Flow Rate = 27 ml/min.			
1.	6.5	0.505	0.495	0.283	8	0.133	0.867	0.294
2.	8	0.642	0.358	0.348	10	0.189	0.811	0.367
3.	9	0.720	0.280	0.392	13	0.316	0.684	0.477
4.	11	0.758	0.242	0.479	18	0.500	0.500	0.661
5.	14	0.838	0.162	0.609	22	0.674	0.326	0.808
6.	20	0.889	0.111	0.870	26	0.736	0,264	0,955
7.	30	0.909	0.091	1.306	38	0.787	0.213	1.095
8.	35	0.932	0.068	1.523	45	0.889	0.111	1.652
9.	45	0.984	0.016	1.958	55	0.944	0.056	2.020
10.	55	1.000	0.000	2.394	65	0.978	0.022	2.387

TABLE 8.

Run 3 ; Temperature = 35°C ; Influent Concentration C_o = 3700 U/ml

Particle diameter =0.1289 cm Bed Height = 7 cm. Flow Rate = 11 ml/min.					Particle diameter = 0.0562 cm Bed Height = 7 cm Flow Rate = 13 ml/min.			
No.	Time (Min.)	$x = C/C_o$	1-x	z	Time (Min.)	$x = C/C_o$	1-x	z
1.	5	0.027	0.973	0.077	9	0.020	0.980	0.165
2.	8	0.068	0.932	0.124	14	0.027	0.973	0.257
3.	12	0.095	0.905	0.184	27	0.115	0.885	0.496
4.	18	0.176	0.824	0.277	32	0.202	0.798	0.588
5.	26	0.352	0.648	0.402	35	0.236	0.764	0.643
6.	36	0.534	0.466	0.557	43	0.454	0.546	0.789
7.	46	0.635	0.365	0.711	53	0.595	0.405	0.973
8.	52	0.677	0.323	0.804	59	0.635	0.365	1.083
9.	58	0.700	0.300	0.897	66	0.676	0.324	1.212
10.	70	0.770	0.230	1.082	72	0.717	0.283	1.332
11.	80	0.800	0.200	1.237	82	0.771	0.229	1.516

TABLE 9

Run 4 ; Temperature=35°C; Influent Concentration C_o = 3650 U/ml

Particle diameter =0.1289 cm Bed Height = 12 cm Flow Rate = 9 ml/min.					Particle diameter = 0.0562 cm. Bed Height = 13 cm. Flow Rate = 9 ml/min.			
1.	32	0.007	0.993	2.336	35	0.000	1.000	2.376
2.	51	0.041	0.959	3.724	45	0.007	0.993	3.055
3.	80	0.189	0.811	5.841	76	0.020	0.980	5.159
4.	96	0.322	0.678	7.009	105	0.090	0.910	7.107
5.	106	0.410	0.590	7.739	115	0.140	0.860	7.786
6.	120	0.610	0.390	8.761	135	0.260	0.740	9.144
7.	140	0.792	0.208	10.221	155	0.660	0.340	10.501
8.	160	0.920	0.080	11.682	185	0.820	0.180	12.538
9.	180	0.972	0.028	13.142	195	0.840	0.160	13.217

TABLE 10

Run 5 ; Temperature = 35°C ; Influent Concentration C_o = 3700 U/ml

Particle diameter=0.1289 cm Bed Height = 12 cm. Flow Rate = 6 ml/min.					Particle diameter=0.0562 cm. Bed Height = 13 cm. Flow Rate = 6 ml/min.			
No.	Time (Min.)	$x = C/C_o$	1-x	z	Time (Min.)	$x = C/C_o$	1-x	z
1.	45	0.010	0.990	2.214	60	0.005	0.995	2.737
2.	65	0.040	0.960	3.198	80	0.010	0.990	3.650
3.	90	0.105	0.895	4.428	100	0.015	0.985	4.562
4.	110	0.185	0.815	5.412	120	0.030	0.970	5.474
5.	130	0.275	0.725	6.396	150	0.075	0.925	6.843
6.	160	0.550	0.450	7.872	180	0.185	0.815	8.212
7.	190	0.820	0.180	9.348	200	0.290	0.710	9.124
8.	200	0.875	0.125	9.840	230	0.610	0.390	10.493
9.	210	0.900	0.100	10.332	240	0.690	0.310	10.949
10.	220	0.930	0.070	10.834	250	0.730	0.270	11.405

TABLE 11

DETERMINATION OF SLOPE AND INTERCEPT

No.	Run (Col.No.)	Flow Rate (ml/min.)	Flow Rate (Cm/min.)	Capacity of the Bed(meq)	Equation Applicable $\ln(1-x) = -mz + I$		
					(m)	(I)	
1.	1(1)	20	12.07	4.27	1.64	-0.5	Bed Ht.=7 cm. Particle diameter=0.1289
2.	2(1)	32	19.3	4.27	1.64	-0.5	
3.	3(1)	11	6.64	4.27	1.70	0.2089	"
4.	4(1)	9	5.43	7.26	0.4367	2.7900	Bed Ht.= 12 cm. Particle diameter=0.1289c
5.	5(1)	6	3.62	7.26	0.440	2.25	"
6.	1(2)	20	12.13	4.38	1.6944	-0.053	Bed Ht.= 7 cm. Particle diameter=0.0562c
7.	2(2)	27	16.4	4.38	1.652	0.3252	"
8.	3(2)	13	7.88	4.38	1.649	0.6883	"
9.	4(2)	9	5.46	8.06	0.4354	3.6422	Bed Ht.=13 cm. Particle diameter=0.0562cm
10.	5(2)	6	3.64	8.06	0.4354	3.6422	"

TABLE 12

LEAST SQUARES REGRESSION MODEL

No.	True I	Estimate I	Residual	Residual sum of squares
1.	-0.5000	-0.5694	0.0694	
2.	-0.5000	-0.2529	-0.2471	
3.	0.2089	-0.0156	0.2244	
4.	2.7900	2.5040	0.2862	
5.	2.2500	2.5830	-0.3329	= 0.9685
6.	-0.0530	0.3331	-0.3861	
7.	0.3252	0.1485	0.1767	
8.	0.6883	0.5178	0.1705	
9.	3.6420	3.5830	-0.0590	
10.	3.6420	3.6620	-0.0201	

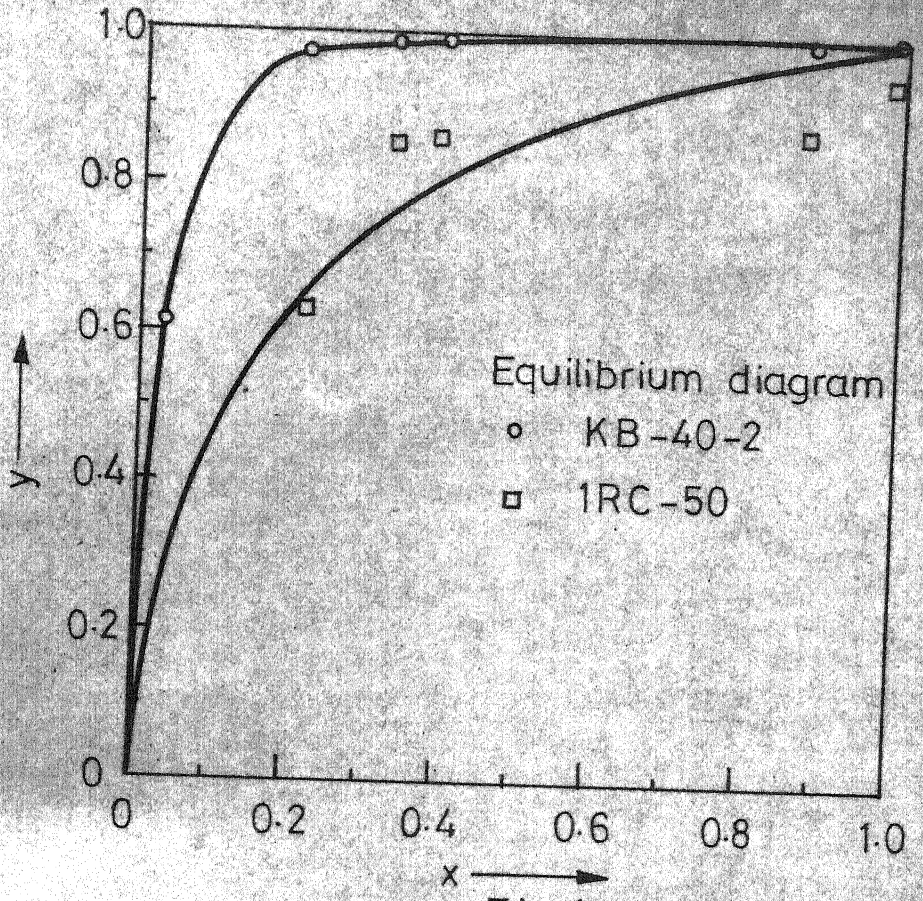

Fig. 4.

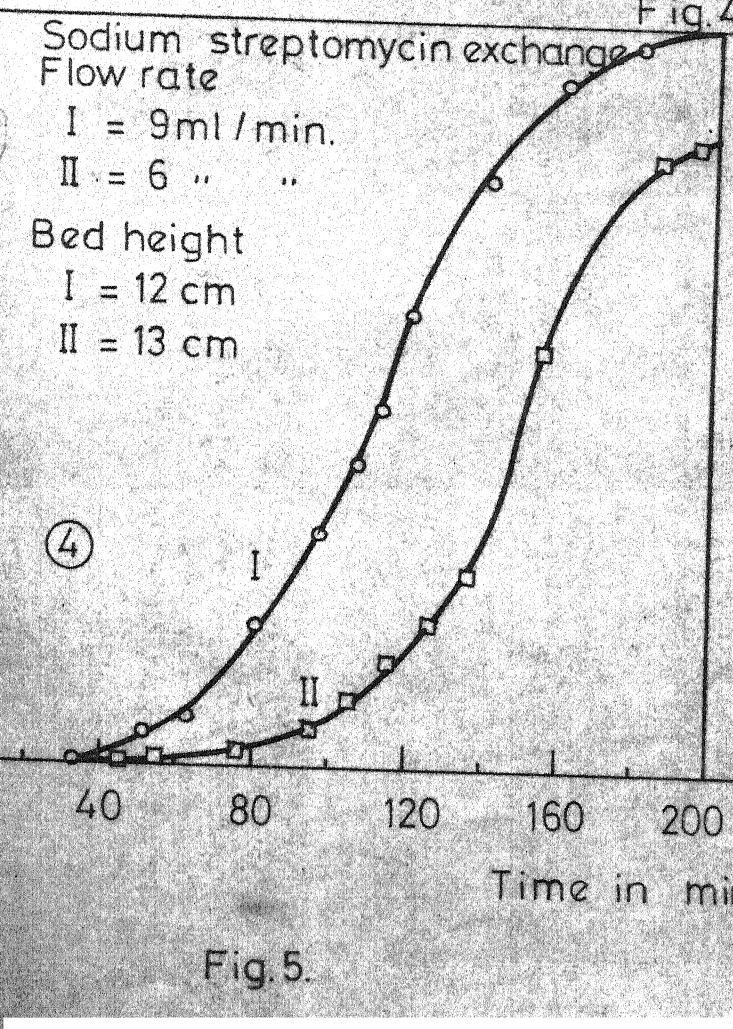

Fig. 5.

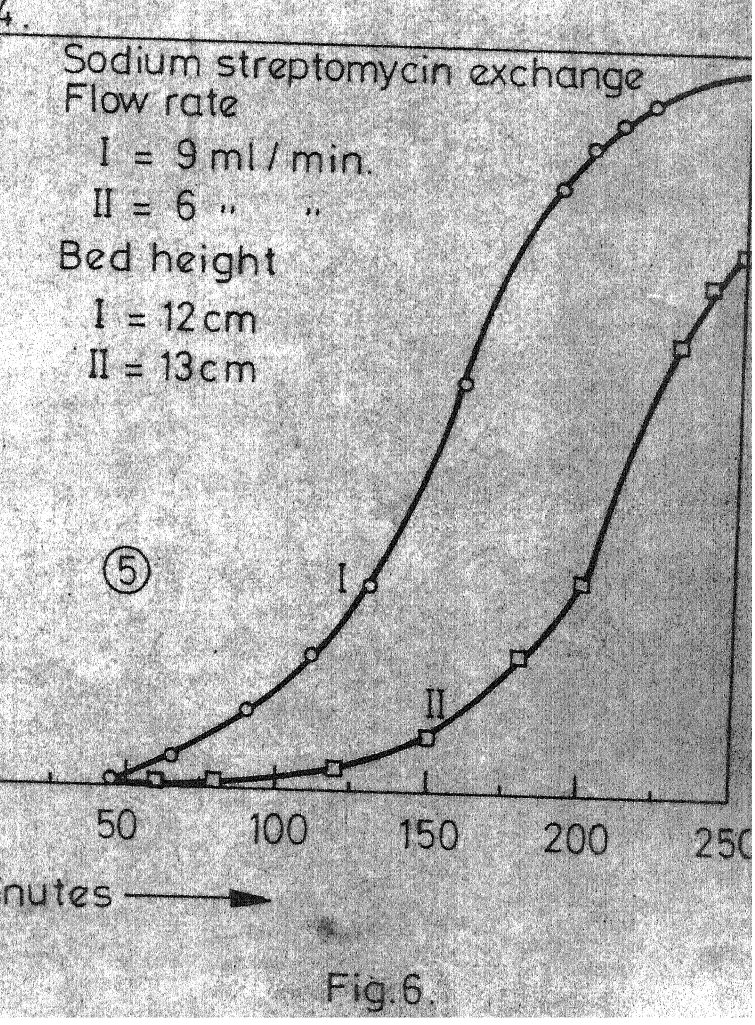

Fig. 6.

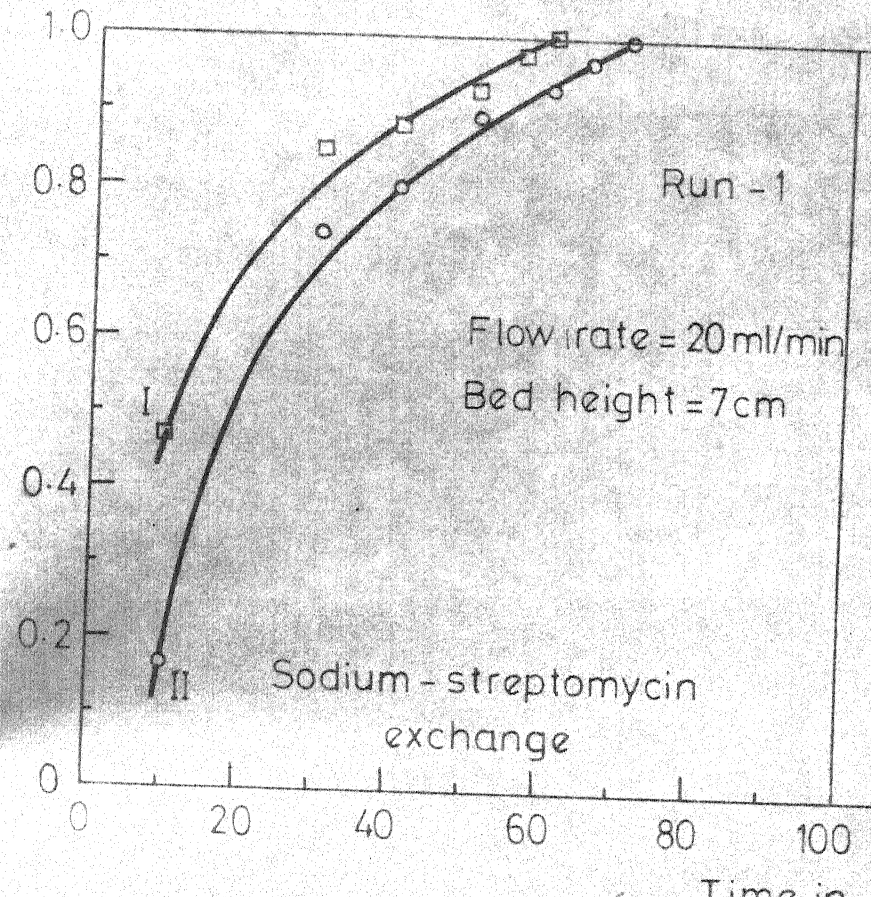

Fig. 7.

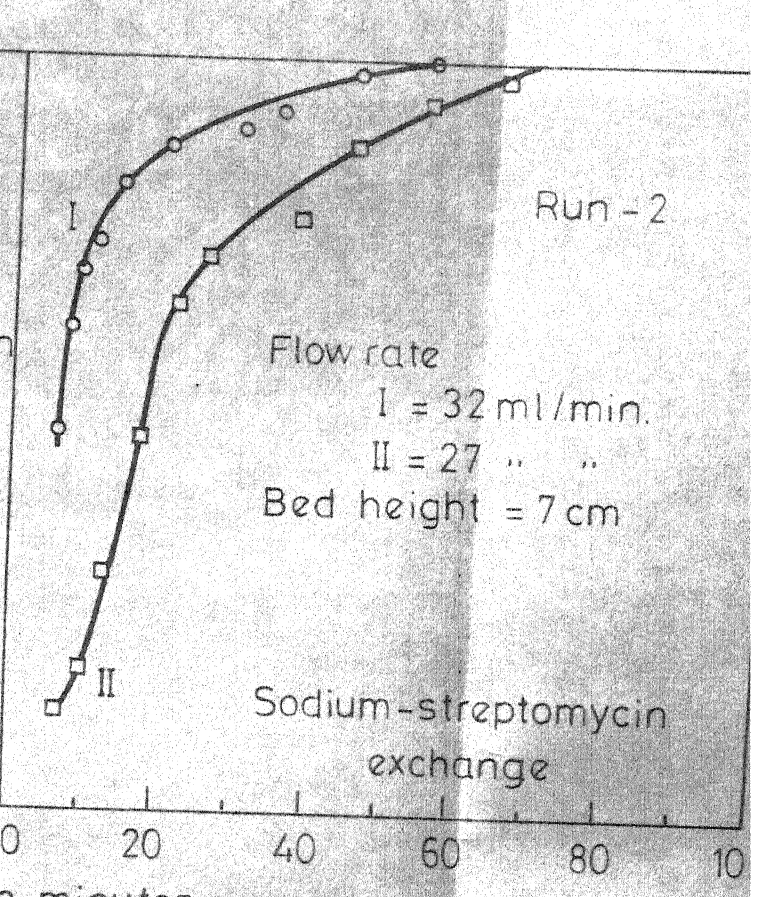

Fig. 8.

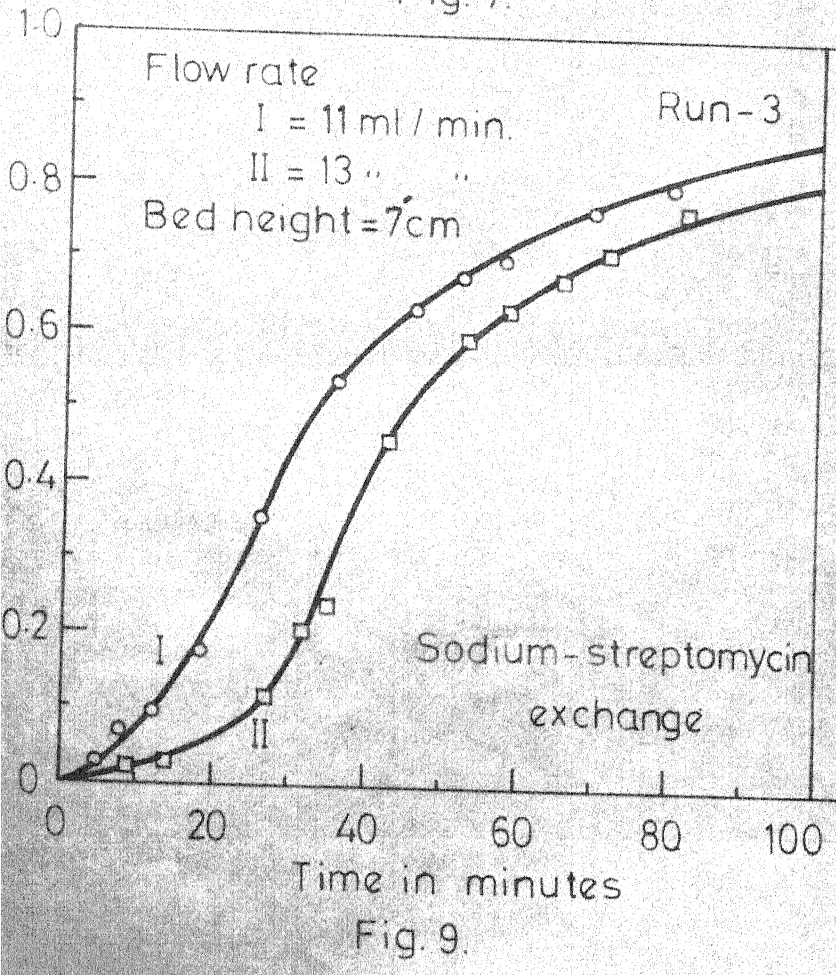

Fig. 9.

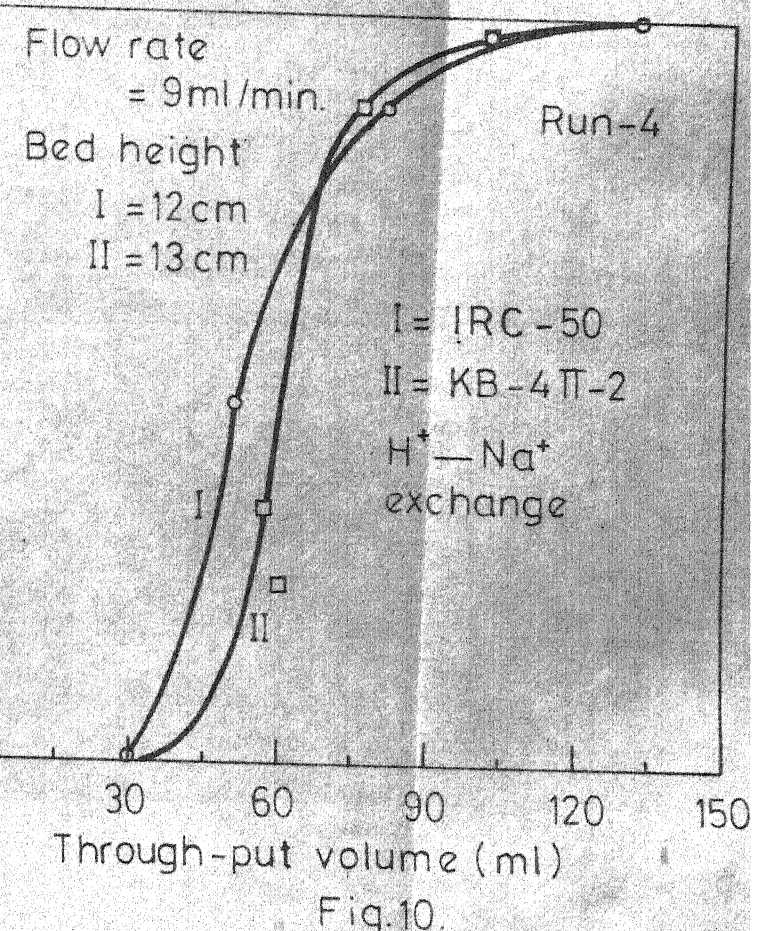

Fig. 10.

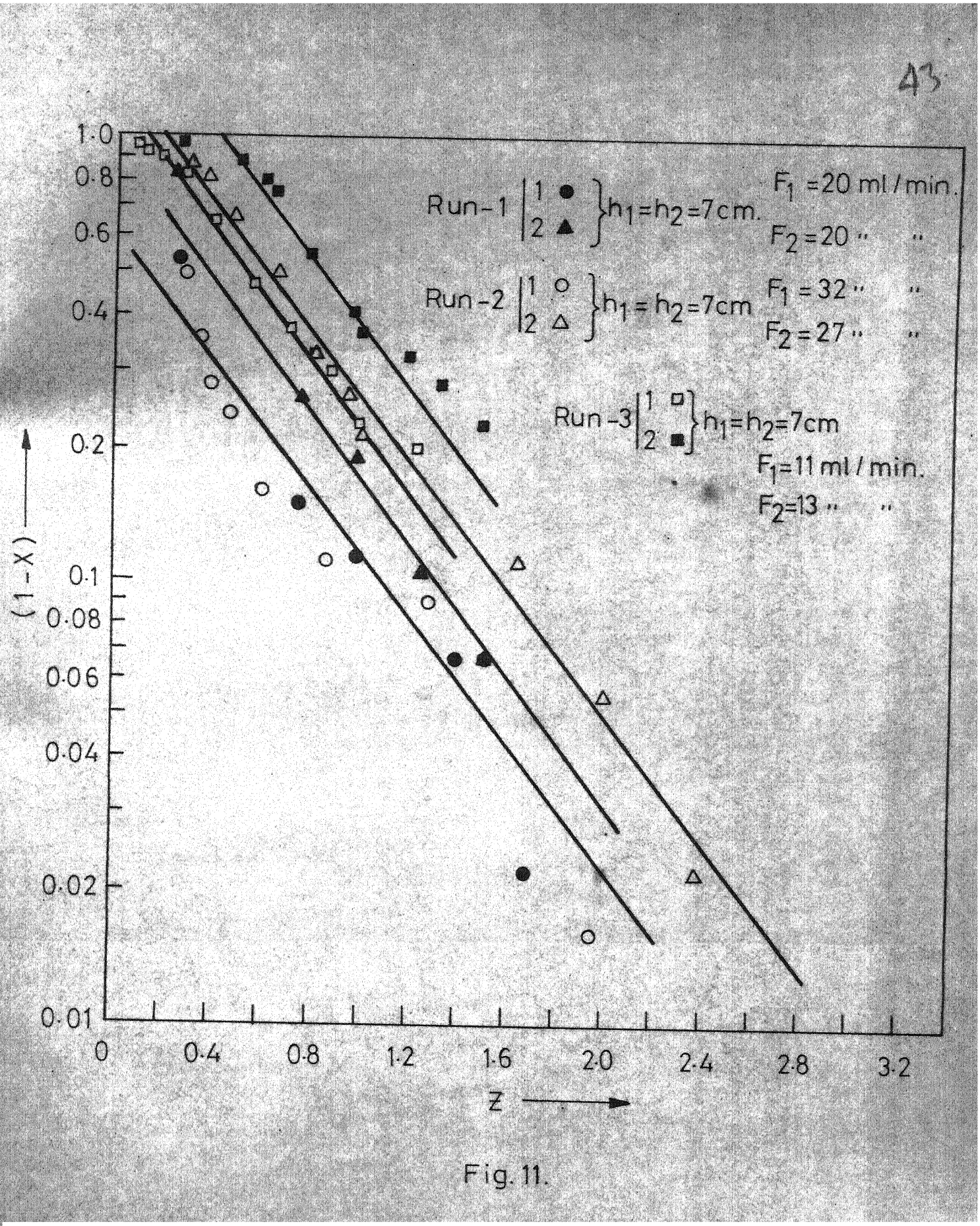
Run-1 1 ● 2 ▲ } h1=h2=7cm.
F1 =20 ml/min.
F2=20 " "
Run-2 1 ○ 2 △ } h1=h2=7cm
F1=32 " "
F2=27 " "
Run-3 1 □ 2 ■ } h1=h2=7cm
F1=11 ml/min.
F2=13 " "
(1-X)
1·0
0·8
0·6
0·4
0·2
0·1
0·08
0·06
0·04
0·02
0·01
0
0·4
0·8
1·2
1·6
2·0
2·4
2·8
3·2
Z

Fig. 11.

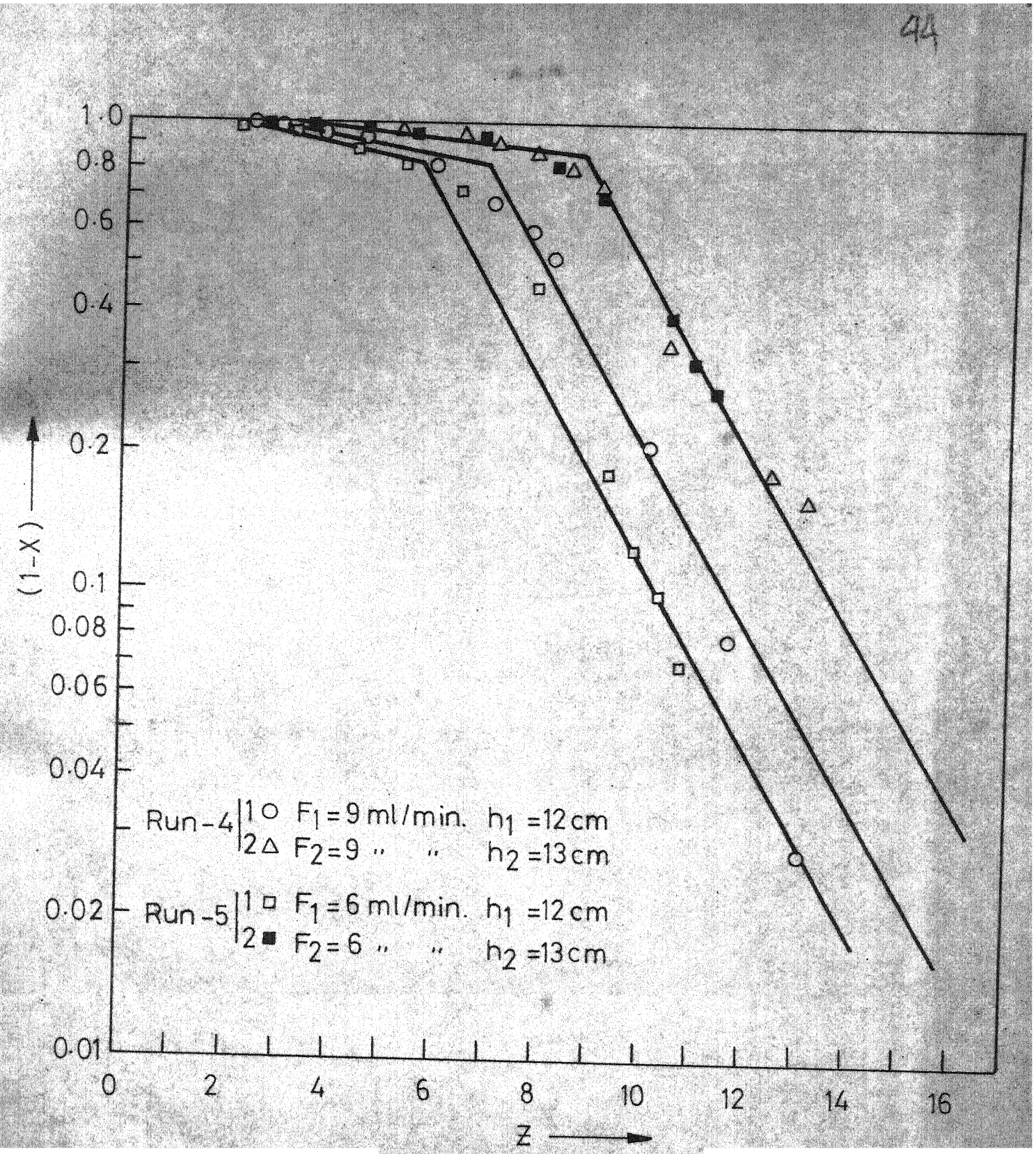
1·0
0·8
0·6
0·4
0·2
0·1
0·08
0·06
0·04
0·02
0·01
(1-X)
Run-4 1 ○ F1 = 9 ml/min. h1 = 12 cm
2 △ F2 = 9 " " h2 = 13 cm
Run-5 1 □ F1 = 6 ml/min. h1 = 12 cm
2 ■ F2 = 6 " " h2 = 13 cm
0
2
4
6
8
10
12
14
16
Z

Fig. 12.

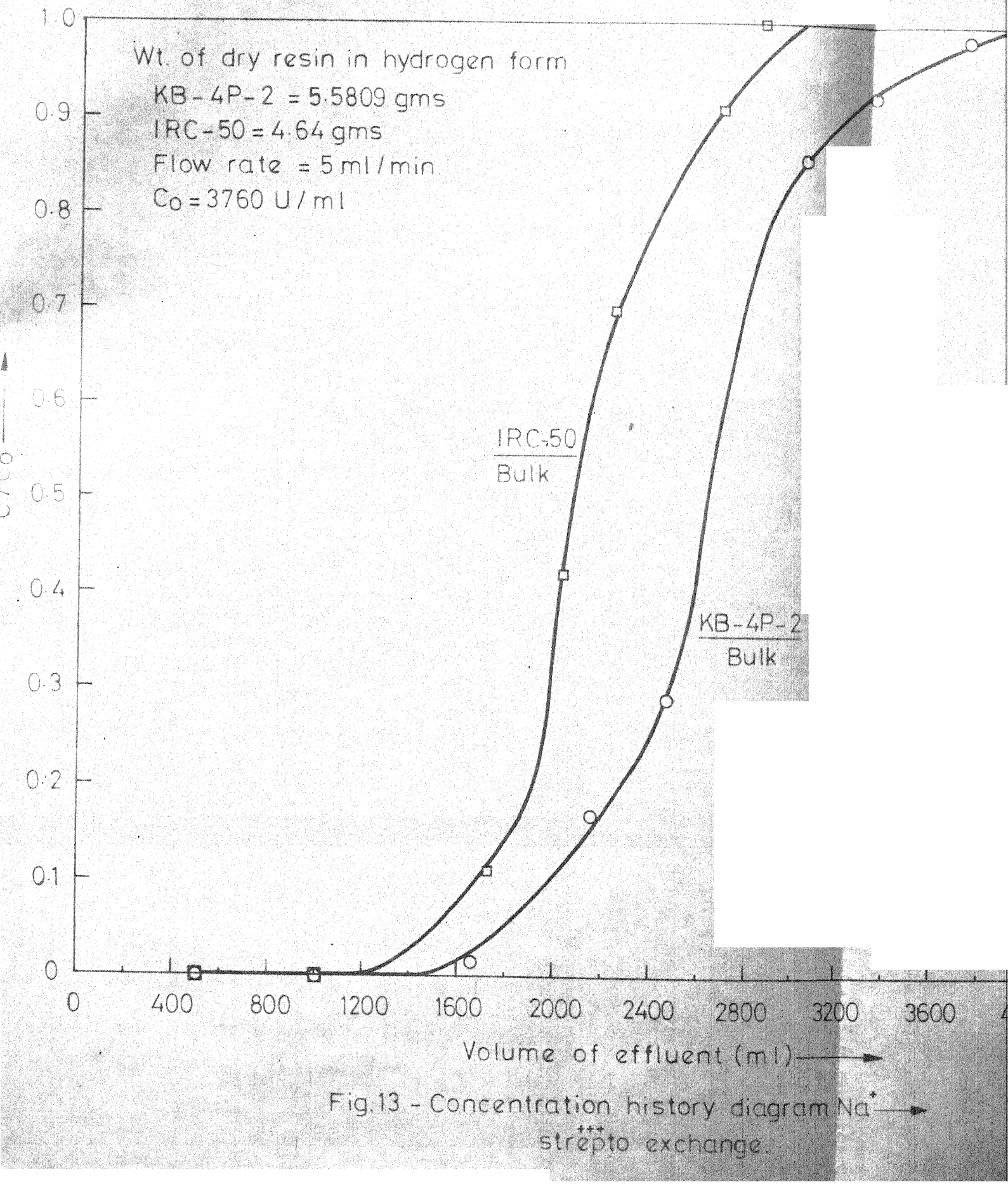

Fig.13 - Concentration history diagram $Na^{+} \longrightarrow strepto^{+++}$ exchange.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The present study showed that streptomycin could be recovered from aqueous solutions by ion exchange technique. Both KB-4P-2 and IRC-50 are suitable for the purpose. However, the Russian resin KB-4P-2 has better capacity than the American resin IRC-50, even though its mechanical properties are slightly inferior. This slight advantage is offset by higher regeneration efficiency shown by the American resin. Care should be taken in substituting proper resin capacity in the design equation for the fixed bed.

The resin capacity in streptomycin form is only ten per cent of its capacity in other cationic forms such as sodium and hydrogen. This fact as well as the regeneration efficiency should be taken into account while designing the fixed beds.

Further work is necessary in correlating the equilibrium data. Also some more data need to be obtained at higher anion concentrations of the equilibrating solutions. The effect of pH on the equilibria, elution and regeneration needs further studies. If a weak cation exchange resin such as Zeokarb 226 is developed in India, work with that also will be necessary before its commercial exploitation.

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REFERENCES

1. Schatz, A. and Waksman, A., Proc. Soc. Exptl. Bio. Med., 55, 66-9 (1944).
2. Bruns, B.P., Antibiotiki, 15(8), 722-7 (1970).
3. Fujiwara, H., Amn. Repts. Takeda Res. Lab. 14, 23-8 (1955).
4. Porter, R.W., Chem. Engg. No.53, pp 94 (Oct. 1946).
5. Ion Exchange Technology, N. Schubert, Chapter Pharmaceuticals, Academic Press, New York,(1956).
6. Kotula, Kunstharz - Ione naustauscher, Plenar-Diskussions Vortr. Symp. 1968 (Pub 1970) pp 415-23.
7. Taylor, US 2528188, October 31, 1950.
8. Howe and Putter, US 2541320, Feb. 13, 1951.
9. Howe and Putter, US 25-41420, Feb. 13, 1951.
10. Peck, R.L.; US 2540284, Feb. 6, 1951.
11. Pfizer Chas and Co. Ltd., Fr. 1518916 (Cl C12d), March 29, 1968.
12. Katarzyna, Pol 56365 (ClA61k), Nov. 30, 1968.
13. Nager Urs, US 3451992 (Cl 260-210, C07c), June 24,1969.
14. Nager Urs,Ger Offen 1916187 (Cl C07d) Oct.8, 1970.
15. Nitealia, Rom 52387 (ClA61k), Oct. 21, 1970.
16. Braune and Werner, Ger (East) 76500 (Cl C07f), Oct.5,1970.
17. Erik, US 3660279 (Cl210-19 C02b), May 2, 1972.
18. Yudin, A.S., USSR 256942 (Cl C07c, A61k), Feb.24,1972.
19. Shiro Shiratsuchi, Japan 5196, Oct.9, 1953.
20. Takagi, Japan 2997, May 29, 1954.
21. Kubota, Japan 5248, August 21, 1954.
22. Strong W.A., J. Biol. Chem. 160, 337-42 (1945).
23. Cartland,G.F., J. Biol. Chem., 165, 463-8 (1950).

24. Reports of Science Research Institute (Japan), 29, 451-6 (1953).

25. Rikagaku, Ken kyusho Hokoku, 38, 95-100 (1962).

26. Boiko I.D., Med Prom SSSR, 16(7), 18-25 (1962).

27. Savitskaya and Bruns, Kolloid Zh 25, 6671 (1963).

28. Savitskaya., Chem-Abs No.3927, Vol.58 (1963).

29. Belaya, S.F., Tr. Leningrad Khim Farm. Inst. 25, 147-63 (1968).

30. Kirk Othmer Encyclo. Chem.Techno. 2nd Ed. 19, 33-98(1969).

31. Ermakov, Khim.Farm. Zh. 3(11), 40-2 (1969).

32. Bogatskii, Antibiotiki 15(5),406-11 (1970).

33. Bruns, B.P., Khim. Farm. Zh. 5(10), 36-40.(1971).

34. Stremovskii, Khim. Farm. Zh. 5(11), 24-30 (1971).

35. Belaya,S.F. , Kolloid Zh. 33(5), 645-7 (1971).

36. Kotula, Kunstharz - Ione-Naus-Tauscher, Plenar-Diskussions Vortr Symp. 1968 (Pub.1970),pp 413-23(Ger)

37. Farm. Pol. 27(1), 17-21, (1971).

38. Spielman, J.Am.Chem.Soc. 67, 2276-7 (1945).

39. Soloman, I.A. J. Biol. Chem. 165, 631-8 (1946).

40. John Seudi et al.,Science 104,486-7 (1946).

41. Titus and Fried, J. Biol.Chem. 168, 393-4.(1947).

42. Federal Register, 12, 2215-7, April 4, 1947.

43. Blauchard and Buhle, J.Pharmacol. Exptl. Ther. 90, 367-74 (1947).

44. Roux, A., Ann. Pharm. Franz, 6, 567-8 (1948).

45. Buck and Mader, Biol. Col. Quim. Puerto Rico, 6, 6-7 (1949).

46. Eisen and Bricker Anal. Chem. 21, 1507-8 (1949).

47. Con and Norman, J. Clin. Invest. 28, 837-9 (1949).

48. Davies, et.al., J. Clin. Invest. 28, 840-2 (1949).

49. Delaby and Stephan, Ann. Pharm. Franz. 8, 513-9 (1950).

50. Kaern, M., Dansk. Tids. Pharm. 25, 21-7 (1951).

51. Valedinskaya, T. Med. Akad. Nauk. SSSR, Antibiotiki ikh Prumenierie 22(1), 82-4 (1952).

52. Monastero, J. Am.Pharm. Assoc. 41, 322-4 (1952).

53. Kartseya and Savitskaya, Zhur. Anal. Khim. 8, 46-9(1953).

54. Szaffir and Bannett, Science 117, 717-18 (1953).

55. Barilari, E.M.,Bioquim. Quim. Farm., 89-95 (1969).(Spanish)

56. Spooner and Douglas, Klotz, Chem. Rev., 39, 241 (1946).

57. Wicke, Kolloid Zh., 167, 289 (1939).

58. Glueckauf and Coates,J. Chem. Soc., 1315 (1947).

59. Glueckauf and Coates,Trans. Faraday Soc., 51, 1540(1955).

60. Vermeulen, Ind. Eng.Chem., 45, 1664 (1953).

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A.1 List of Companies Manufacturing Streptomycin in India

1. M/S Alembic Chemical Works Ltd.
   Alembic Road
   Baroda-3

2. M/S Dey's MedicalStores (Mfg) Pvt. Ltd.,
   62, Bondel Road
   Calcutta-19

3. M/S Glaxo Laboratories (I) Private Ltd.,
   Aligarh, Bombay and Thana

4. M/S Hindustan Antibiotics Limited
   Pimpri, Poona-18

5. M/S Indian Drugs and Pharmaceuticals Ltd.
   Antibiotics Project
   Virbhadra

6. M/S May and Baker Limited
   Bhandup, Bombay-78

7. M/S Merck Sharp and Dohme of India Ltd.
   Nahur Village
   Bhandup
   Bombay-78

8. M/S Pfizer Limited
   Power Works
   Bunder, Darukhana
   Bombay-1

9. M/S Sarabhai Chemicals
   Wadi Wadi
   P.B. No.31
   Baroda-1

A.2 Column Parameters

1. Internal dia of column 1 = 1.452 cm.
2. Internal dia of column 2 = 1.450 cm.

Cross sectional area of column 1 = 1.656 cm^2

Cross sectional area of column 2 = 1.648 cm^2

A.3 Void Fraction in Packed Beds

1. Average particle diameter in column 1 = 0.1289 cm.

$$\frac{\text{Particle diameter}}{\text{Tube diameter}} = \frac{0.1289}{1.452} = .088$$

$$\text{Fraction voids}_{1} = 0.331 \qquad (56)$$

2. Average particle diameter in column 2 = 0.0562 cm

$$\frac{\text{Particle diameter}}{\text{Tube diameter}} = \frac{0.0562}{1.450} = 0.0387$$

$$\text{Fraction voids}_{2} = 0.32$$

A.4 Swelling Data H^+R - Na^+R

1. Resin: KB-4P-2

Volume of resin in hydrogen form (m) a	Volume of resin in sodium form (m) b	Percent swelling $\frac{b-a}{a} * 100$
8.5	19.2(at pH 10)	$\frac{10.7}{8.5} * 100 = 125$
10.3	24.2(at pH 10)	$\frac{13.9}{10.3} * 100 = 135$

Average % swelling = 130%

2. Resin: IRC-50

16.1	24.1	$\frac{8.0}{16.1} * 100 = 50$ at pH = 12
16.1	25.1	$\frac{9.0}{16.1} * 100 = 56.2$ at pH = 8

Observations: From hydrogen form to sodium form of exchange, both the resins increase in size. But for sodium form to streptomycin form of exchange KB-4 P-2 shrinks whereas IRC-50 swells a little. However shrinking data were not recorded.

A.5 Bulk Density Determination:

1. KB-4 P-2 sodium form

Amount of wet resin = 10.2220 gms

Volume of the resin = 14.6 m (at pH=10)

$\therefore$ Density = $\frac{10.222}{14.6}$ = 0.7 gms/ml

2. KB-4 P-2 Hydrogen form:

Volumeof wet resin (ml) a	Amount of wet resin (gms) b	Density gms/ml b/a
14.076	9.416	0.67
16.9744	11.81	0.695
Average		0.6825

A.6 Sample Calculations

1. Water Content Determination:

a. Resin KB-4P-2 in Sodium Form:

No.	Weight of wet Resin (gm) A	Weight of dried resin (gm) B	Water Content 100(1-B/A)
1.	2.5230	0.7515	70.25
2.	3.2915	0.9870	69.9
	Average water content = 69.65%		
1.	6.3615	1.8625	70.8
2.	13.9725	4.028	71.2
3.	7.3435	2.1255	71.0
	Average water content = 71%		

b. Resin KB-4P-2 in Hydrogen Form:

1.	3.1045	1.6610	46.5
2.	2.6950	1.4650	45.5
	Average water content = 46%		

c. Resin IRC-50 in Sodium Form:

1.	4.9550	1.5815	68.0
2.	3.9690	1.2760	68.8
3.	2.9400	0.9705	67.0
	Average water content = 68%		
1.	5.8655	1.8980	67.7
2.	4.0345	1.3105	67.5
3.	3.7600	1.2190	67.6
	Average water content = 67.6%		

d. Resin IRC-50 in Hydrogen Form:

No.	Weight of wet Resin (gm.)	Weight of dried Resin (gm.)	Water content 100(1-B/A)
1.	5.5265	2.2905	58.56
2.	13.9110	7.1690	48.48

Average water content = 53.52%

2. Capacity Determination:

a. Resin IRC-50 in Hydrogen Form to Sodium Form:

Amount of dry resin in the column = 4.64 gms.

Concentration of sodium ions in influent solution = 1.005N

Volume of solution passed through the column = 165 ml

Total sodium passed through the column = 165x1.005=165.825 m.equiv.

Concentration of effluent solution = $\frac{3.5}{5}$x1.005 =0.7 meq/ml.

Total amount of sodium in effluent = 165x0.7=115.5 meq

Amount of sodium adsorbed = 165.825 - 115.5
= 50.325 meq

$\therefore$ Capacity = $\frac{50.325}{4.64}$ = 10.9 m eq./gm.dry resin in hydrogen form

b. Resin KB-4P-2 in Hydrogen Form to Sodium Form:

Amount of dry resin in the column = 5.5809 gms.

Total effluent collected = 200 ml

5 ml sample uses 3.2 ml 1.005N oxalic acid

$\therefore$ 200 ml sample uses $\frac{3.2}{5}$ x 100 = 128 ml oxalic acid

Sodium adsorbed = (200-128)x 1.005 = 72.36 meq.

$\therefore$ Capacity = $\frac{720.36}{5.5809}$ = 12.9 $\frac{\text{mequivalents}}{\text{gm.dry resin in hydrogen form}}$

c. Resin KB-4P-2 in Sodium Form to Streptomycin Form:

Amount of dry resin in the column = 7.308 gm.

Volume of elute (at pH 5.4) = 1120 ml

Transmittance of elute sample = 6.75%

Concentration of elute sample = 7040 U/ml

$\therefore$ Capacity $= \frac{7040 \times 1120}{7.308} = 1077{,}000$ U/gm dry resin in sodium form

$= \frac{1077000}{800{,}000} \times \frac{1000}{1457.4} \times 2$

$= 1.8475$ m.eq/gm.dry resin in sodium form

For the same run (Resin in wet condition)

Amount of wet resin in the column = 24.36 gms.

$\therefore$ Capacity $= \frac{7040 \times 1120}{24.36} = 323{,}700$ U/gm.wet resin in sodium form

$= \frac{323700}{800{,}000} \times \frac{1000}{1457.4} \times 2 = 0.5555 \frac{\text{meq}}{\text{gm.wet resin in sodium form}}$

3. Equilibrium Calculations

Resin - IRC-50, Capacity = 2.4008 meq/gm dry resin in H^+ form

= 1403560 U/gm dry resin in H^+ form

Composition of the equilibrating solution		$x = \frac{2b}{2b+a}$	Amount of Streptomycin in resin per gm dry hydrogen form	
Sodium a	Streptomycin b		c	$y = \frac{c}{1403560}$
1/90.346 = 0.01168 m.moles/gm solvent	4360 U/ml = .00374 m.moles/gm.solvent	0.39	1205,445	0.8588

4. Through Put Parameter:

The distribution coefficient D_E has been defined as

$$D_E = \frac{QW}{C_o \; v \in}$$

Throughput parameter Z is defined below:

$$ZZ = \frac{T}{D_E \; v \; /F} = \frac{T}{\frac{QW}{C_o v \in} \cdot v \in /F} = \frac{C_o \; FT}{QW}$$

The values of throughput parameter have been calculated for all the runs and included in the kinetic data tables

Sample Calculation: (For Run 3)

$C_o = \frac{3700 \text{ x } 1000}{800000 \text{ x} 728.7}$ meq/gm solution

Q = 0.5555 meq/gm. wet resin sodium form

F = 11 ml /min.

W = 8.1144 gms wet resin in sodium form

Z = .0064 x 11 T / 0.5555 x 8.1144

= 0.01546 T

A.7 Breakthrough Data

Sodium - Streptomycin Exchange

Temperature = 37°C

Flow Rates = 5 ml/min.

Influent Concentration = C_o = 3760 U/ml = 0.0064 meq/gm solution

Resin in KB-4P-2			IRC - 50		
Amount = 5.5809 gm hydrogen form (dry) = 7.31 gm dry sodium form			4.64 gm hydrogen form (dry)		
Volume passed (ml)	Concentration (U/ml)	$x = \frac{c}{C_o}$	Volume passed (ml)	Concentration (U/ml)	$x = \frac{c}{C_o}$
1660	50	0.013	1000	0	0
2150	630	0.169	1530	420	0.112
2460	1100	0.292	2030	1580	0.420
3020	3200	0.851	2240	2620	0.697
3360	3480	0.952	2660	3420	0.909
3750	3700	0.984	2830	3760	1.000

From Fig. we see that

Total capacity = $\frac{2700 \times 3760}{5.5809}$ = 1800,000 U/gm dry resin H^+ form

$= \frac{2100 \times 3760}{4.64}$ = 1700,000 U/gm. dry resin H^+ form

Eluted Streptomycin

= 1426,000 U/gm dry resin H^+ form

= 1403,000 U/gm dry resin in H^+ form

∴Percent Recovery

$= \frac{1426000}{1800000} \times 100 = 79.2\%$

$= \frac{1403000}{1700000} \times 100 = 82.5\%$

NOMENCLATURE

C	Concentration in effluent
C_o	Concentration in influent
q	Equilibrium concentration in resin phase
Q	Capacity of the resin
x	c/C_o
y	q/Q
Y_r	Dimensionless solid phase concentration at any internal radius
r	radius of the resinparticles
d_p	Average diameter of resin particles
h	Height of fixed bed
F	Flow rate of influent
T	Time
I	Intercept of the plot between ln(1-x) and Z
m	Slope of the plot between ln(1-x) and Z
W	Weight of the resin in the bed
	Void fraction of the bed
v	Packed volume of resin bed.
D_p	Particle phase diffusivity
D_E	Distribution coefficient
Z	Throughput parameter
N_{PF}	Number of transfer units based on particle phase
N_{fF}	Number of transfer units based on fluid phase

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